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# A New Species of the *Fejervarya limnocharis* Complex from Japan (Anura, Dicroglossidae)

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We describe a new species of dicroglossid frog of the *Fejervarya limnocharis* complex from western Honshu, Japan Mainland. The new species, *Fejervarya kawamurai*, is genetically closer to *F. sakishimensis* than to *F. limnocharis*. It differs from *F. sakishimensis* by smaller tympanum, head, forelimb, hindlimb, foot, and tibia lengths, all relative to snout-vent length, and from *F. multistriata* by relatively shorter forelimb, hindlimb, foot, and tibia. From *F. limnocharis* and *F. iskandari*, it is differentiated by relatively smaller forelimb, hindlimb, foot, and tibia lengths. Taxonomic problems of *Fejervarya* populations occurring in Central Ryukyus, continental China, and Taiwan are discussed.

**Key words:** *Fejervarya*, new species, cryptic species, East Asia, Japan Mainland, Ryukyu, China, Taiwan, taxonomy

## INTRODUCTION

Asian dicroglossid frogs related to *Fejervarya limnocharis* (Gravenhorst, 1829) are wide-ranging and notoriously difficult to classify taxonomically (Matsui et al., 2007). Recent extensive studies in this genus revealed the presence of two distinct genetic groups; i.e., South Asian and Southeast Asian groups (Sumida et al., 2007; Kotaki et al., 2010). The Southeast Asian group includes East Asian taxa and systematic studies of this group date back to the finding of two distinct populations occurring sympatrically on Java (Toda et al., 1998a), the type locality of *F. limnocharis*. The cryptic Javanese population genetically distinct from *F. limnocharis* was later described as *F. iskandari* Veith, Kosuch, Ohler and Dubois, 2001 (Veith et al., 2001).

Simultaneous studies on the East-Southeast Asian lineage revealed the presence of many cryptic species in this group (Toda et al., 1997, 1998a, b). Among them, the population from the Southern Ryukyus, Japan was noted for its unique genetic features different from the other East Asian congeners (Toda et al., 1997, 1998b) as well as from *F. limnocharis* (Toda et al., 1998a). Matsui et al. (2007) morphologically compared the population with topotypic specimens of *F. limnocharis*, *F. multistriata* (Hallowell, 1861), and *F. iskandari*, and by combining the results obtained with known genetic uniqueness (Toda et al., 1997, 1998a, b),

they described the Southern Ryukyu populations as a distinct species, *F. sakishimensis* Matsui, Toda, and Ota, 2007.

By contrast, Matsui et al. (2007) left taxonomic problems of the populations from Japan Mainland, the Central Ryukyus, continental China, and Taiwan for future studies. Of these, at least the population from the Japanese Mainland differs substantially from *F. limnocharis* (Djong et al., 2007a, b). It is also sufficiently different from *F. sakishimensis* in morphological, acoustic, and genetic characteristics (Toda et al., 1997; Matsui et al., 2007). Taxonomic positions of the remaining populations are less clear, but the available genetic evidence indicates conspecific status of the Central Ryukyus (Nishioka and Sumida, 1990; Toda et al., 1997; but see Sumida et al., 2007) and some Chinese (Toda et al., 1997) populations with the population from mainland Japan (see discussion). This study is aimed to provide a description of the unnamed population from mainland Japan so as to facilitate future studies of the surrounding populations.

## MATERIALS AND METHODS

A total of 116 preserved specimens of the *F. limnocharis* complex from East and Southeast Asia stored at the Institute for Amphibian Biology, Hiroshima University (IABHU), Graduate School of Human and Environmental Studies, Kyoto University (KUHE), and Department of Zoology, Graduate School of Science, Kyoto University (KUZ) were examined: *Fejervarya* sp. from Hiroshima of Honshu Island (n = 10), Kumamoto of Kyushu Island (n = 10), Amamioshima, Tokunoshima, and Yoronjima Islands of the Amami Group (n = 12), and Okinawajima Island of the Okinawa Group (n = 7); *F. sakishimensis* from Ishigakijima and Iriomotejima Islands of the Yaeyama Group (n = 38); *F. limnocharis* from Malinping, Java (n = 8); *F. iskandari* from Malinping, Java (n = 10); *F. multistriata*

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from Hongkong ( $n = 9$ ) and Guangzhou, southeastern China ( $n = 12$ ). In order to assess morphometric differences among the population samples, we took following 14 body measurements to the nearest 0.1 mm with dial calipers, or with a stereoscopic binocular microscope, mainly after Matsui (1984): (1) snout-vent length (SVL); (2) head length (HL); (3) snout length (SL); (4) eye length (EL); (5) tympanum diameter (TD); (6) head width (HW); (7) internarial distance (IND); (8) interorbital distance (IOD); (9) upper eyelid width (UEW); (10) forelimb length (FLL); (11) hindlimb length (HLL); (12) tibia length (TL); (13) foot length (FL), and (14) inner metatarsal tubercle length (IMTL). Data for species other than *Fejervarya* sp. are those already used by Matsui et al. (2007).

We adopted the system used by Savage (1975) for description of toe webbing states. We examined variation in adult SVL by analysis-of-variance (ANOVA) with the Tukey range test. We converted each value to a percentage ratio to SVL for comparisons of the other characters. We first confirmed the absence of significant differences in each of those characters between sexes in Hiroshima and Kumamoto samples of *Fejervarya* sp. (both entirely consisting of adults), and then combined data for both sexes for comparisons among these and other samples. We performed Kruskal-Wallis tests with nonparametric multiple comparisons or Mann-Whitney U tests for ratio variables to detect the presence or absence of differences in the frequency distributions, setting the significance level at 0.05.

## SYSTEMATICS

### *Fejervarya kawamurai* sp. nov.

(Figs. 1–3)

#### Synonymy

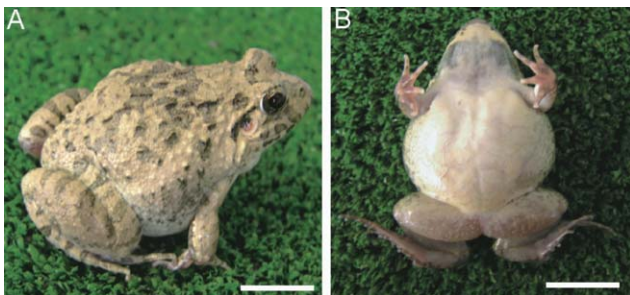
*Rana gracilis* (not of Gravenhorst, 1829): Boulenger, 1882, p. 28 (part); Boettger, 1885, p. 159; Boulenger, 1887, p. 149 (part); Okada, 1891, p. 67; Brown, 1902, p. 185.

*Rana limnocharis*: Boulenger, 1890, p. 450 (part); Fritze, 1894, p. 865; Boettger, 1895, p. 103; Stejneger, 1907, p. 127 (part); Boulenger, 1920, p. 28 (part); Okada, 1930, p. 124 (part); Okada, 1931, p. 138 (part); Inger, 1947, p. 334 (part); Okada, 1966, p. 112 (part); Kuramoto, 1968, p. 109 (part); Nishioka and Sumida, 1990, p. 125 (part); Toda et al., 1997, p. 143 (part); Sumida et al., 2002, p. 293 (part).

*Rana (Rana) limnocharis limnocharis*: Nakamura and Uéno, 1963, p. 49 (part); Kuramoto, 1969, p. 45; Kuramoto, 1971, p. 105.

*Rana (Euphlyctis) limnocharis limnocharis*: Maeda and Matsui, 1989, p. 110 (part).

*Rana (Limnometes) limnocharis*: Maeda and Matsui, 1999, p. 110.



**Fig. 1.** (A) Dorsolateral and (B) ventral views of the male holotype of *Fejervarya kawamurai* (IABHU - F2184, SVL = 40.5 mm) in life. Scale bar, 10 mm.

*Limnometes limnocharis*: Kuramoto and Joshy, 2001, p. 86 (part).

*Fejervarya limnocharis*: Kuramoto, 2006, p. 1 (part); Djong et al., 2007a, p. 360 (part); Djong et al., 2007b, p. 1197 (part); Sumida et al., 2007, p. 547 (part); Kotaki et al., 2010, p. 387.

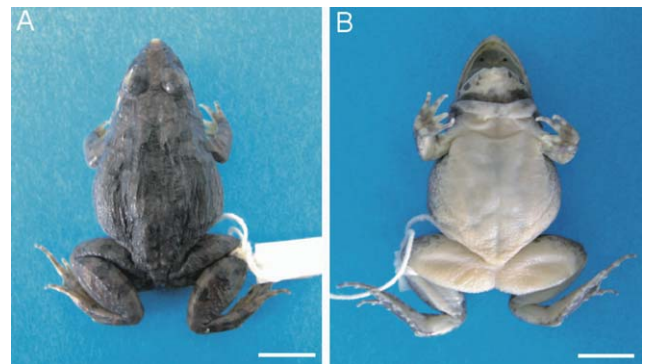
*Fejervarya multistriata* Fei et al., 2009, p. 1310 (part).

#### Diagnosis

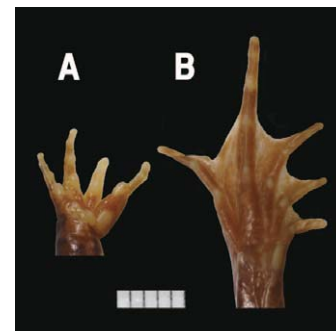
A small-sized species of the *F. limnocharis* complex, with adult SVL 36.8–48.7 mm in females, 30.7–41.8 mm in males (Table 1). This new species is genetically closer to *F. sakishimensis* than to *F. limnocharis*, but is differentiated from *F. sakishimensis* by smaller tympanum, head, forelimb, hindlimb, foot, and tibia lengths, all relative to SVL. It differs from *F. multistriata* by relatively shorter forelimb, hindlimb, foot, and tibia. From *F. limnocharis* and *F. iskandari*, it is differentiated by relatively smaller forelimb, hindlimb, foot, and tibia lengths.

#### Etymology

We dedicate the species name “*kawamura*” to the late Dr. Toshihiro Kawamura, Emeritus Professor of the Institute for Amphibian Biology of Hiroshima University, who was one of the outstanding Japanese herpetologists and the pioneer founder who established the Institute.



**Fig. 2.** (A) Dorsal and (B) ventral views of the male holotype of *Fejervarya kawamurai* (IABHU - F2184) after preservation. Scale bar, 10 mm.



**Fig. 3.** Ventral views of the right hand (A) and foot (B) of the male holotype of *Fejervarya kawamurai* (IABHU - F2184). Scale indicates 5 mm.

**Table 1.** Morphological variation in adult *Fejervarya kawamurai* from Japan Mainland (Hiroshima in Honshu and Kumamoto in Kyushu) and Central Rykyus (Amami Group and Okinawa Group). SVL (mean  $\pm$  1SD) and medians of % ratios of other characters to SVL, followed by ranges in parentheses. See text for character abbreviations.

	Males				Females			
	Hiroshima (n = 5)	Kumamoto (n = 5)	Amami (n = 3)	Okinawa (n = 4)	Hiroshima (n = 5)	Kumamoto (n = 5)	Amami (n = 9)	Okinawa (n = 3)
SVL	36.4 $\pm$ 2.1 (35.8–40.5)	35.0 $\pm$ 4.1 (30.7–40.0)	41.4 $\pm$ 0.3 (41.2–41.8)	35.0 $\pm$ 1.6 (32.7–36.3)	41.1 $\pm$ 2.5 (37.9–44.6)	40.9 $\pm$ 2.3 (37.9–43.2)	45.8 $\pm$ 4.5 (36.8–48.7)	44.0 $\pm$ 1.7 (43.0–45.9)
RHL	39.6 (37.5–41.1)	37.5 (36.4–38.3)	36.7 (35.4–37.3)	38.1 (35.6–40.6)	36.8 (36.5–38.7)	36.9 (34.1–37.5)	37.0 (35.4–39.6)	36.0 (35.5–37.9)
RSL	15.9 (14.8–16.1)	15.3 (15.0–16.6)	16.5 (15.5–17.4)	15.5 (15.0–15.7)	15.2 (14.8–15.6)	15.1 (14.8–16.0)	17.4 (15.5–18.3)	15.3 (15.1–15.5)
REL	14.2 (13.1–14.6)	14.5 (13.7–16.3)	11.7 (11.2–11.9)	14.5 (14.2–14.7)	13.8 (12.8–14.5)	13.7 (12.2–13.9)	11.5 (10.0–12.4)	13.9 (13.5–14.2)
RTD	7.7 (6.9–8.5)	6.9 (6.3–7.2)	6.9 (6.6–7.5)	7.3 (6.3–8.0)	6.8 (6.3–7.3)	7.1 (6.5–7.7)	6.5 (5.9–7.2)	6.3 (6.0–7.2)
RHW	35.4 (33.6–37.1)	33.6 (33.0–36.5)	30.6 (30.3–34.6)	33.9 (32.2–34.4)	35.1 (33.4–35.3)	33.0 (31.9–35.7)	34.7 (33.0–35.9)	34.2 (34.0–34.9)
RIND	7.0 (6.6–8.0)	6.5 (6.3–7.7)	7.7 (7.5–8.5)	7.4 (6.7–8.3)	7.0 (6.3–7.3)	7.1 (6.7–7.4)	7.3 (6.8–8.2)	7.7 (7.0–8.1)
RIOD	5.5 (4.9–6.1)	6.0 (5.3–6.7)	6.3 (5.7–6.5)	5.7 (5.1–6.4)	5.2 (4.8–5.6)	5.1 (4.2–6.1)	5.6 (5.0–6.5)	5.6 (5.0–5.8)
RUEW	7.9 (7.8–8.5)	8.9 (8.1–9.8)	8.4 (7.8–8.7)	9.4 (8.8–10.7)	7.8 (7.5–8.2)	8.7 (7.8–9.3)	8.6 (7.7–9.5)	8.9 (8.6–9.1)
RFL	52.3 (49.2–53.4)	50.4 (47.9–53.0)	50.7 (45.6–57.6)	51.6 (47.0–56.3)	49.2 (37.2–52.4)	50.2 (47.0–52.9)	53.9 (50.0–56.0)	52.6 (52.3–54.9)
RHLL	136.5 (131.3–153.6)	141.8 (134.0–147.9)	141.6 (130.3–157.9)	147.3 (139.0–157.8)	139.0 (126.1–148.6)	138.5 (129.0–146.1)	147.6 (140.9–160.4)	157.7 (154.7–159.5)
RTL	41.3 (39.0–44.8)	43.3 (39.3–43.8)	42.3 (38.3–46.2)	44.0 (42.2–45.9)	41.7 (37.1–43.3)	40.0 (39.3–44.0)	44.0 (43.0–48.5)	45.8 (45.6–47.9)
RFL	46.4 (43.7–52.1)	47.8 (44.7–51.8)	48.3 (45.1–52.5)	49.8 (45.3–52.6)	46.9 (41.9–50.2)	47.0 (43.0–48.6)	49.6 (48.1–53.3)	51.6 (51.0–51.9)
RIMTL	5.3 (4.1–6.2)	5.5 (4.8–5.9)	4.5 (3.9–6.1)	5.3 (5.0–6.0)	5.3 (4.8–6.5)	5.0 (4.4–5.6)	5.0 (4.1–6.6)	5.7 (5.0–6.0)

### Holotype

IABHU - F2184 (Figs. 1–3), an adult male collected from paddy field around 2 km from Hiroshima University, Higashihiroshima-shi, Hiroshima Prefecture, western Honshu, Japan (34° 23' N, 132° 42' E, alt. 200 m a.s.l.) on 30 May 2006 by H. T. Djong and M. Sumida.

### Paratypes

Four males (IABHU - F2185–F2188) and five females (IABHU - F2189–F2193), collection data same as the holotype.

### Referred specimens

Honshu: Higashihiroshima-shi, Hiroshima Pref., five males (IABHU - 18140, 18158, 18686, 18687, 18698) and five females (IABHU - 18159–18162, 18697). Kyushu: Nishihara-mura, Kumamoto Pref., five males (KUHE 43233, 43251, 43264, 43278, 43284) and five females (KUHE 43227, 43228, 43244, 43300, 43302). Central Ryukyus: Kagoshima Pref., Amamioshima Is., one male (KUHE Unnumbered) and two females (KUZ 4616, KUHE Unnumbered); Tokunoshima Is., seven females (KUZ 4040, 4042, 4043, 4045, 4047–4049); Yoronjima Is., two males (KUZ 2947, 2948); Okinawa Pref., Ginowan-shi, Okinawajima

Is., four males (KUZ 30988, 30989, 30993, 31000) and three females (KUZ 30985, 30990, 30996).

### Description of holotype (measurements in millimeters)

Body moderately stocky, SVL 40.5; head triangular, longer (15.2) than wide (13.6); snout dorsally slightly pointed, projecting beyond lower jaw, rounded in profile; eye moderate, length (5.3) shorter than snout (6.0); canthus indistinct; lores slightly concave, sloping; nostril below canthus, slightly closer to eye (2.4) than to tip of snout (3.2); internarial distance (2.7) wider than interorbital distance (2.0); latter much narrower than upper eyelid (3.2); distance between anterior tips of eyes (4.4) less than half of distance between posterior tips of eyes (7.7); pineal spot visible, on line connecting anterior corners of orbits; tympanum (2.8) conspicuous, about half of eye diameter and separated from eye by about one-third of tympanic diameter (0.9); vomerine teeth in short, oblique groups, beginning from line connecting centers of choanae and extending posteromedially, groups more narrowly separated from each other than from choanae; tongue deeply notched, without papillae; median external subgular vocal sacs; vocal openings slit-like, on each side of mouth.

Forelimb (21.2) moderately stout; fingers thin, unwebbed;

second finger with very narrow fringes of skin; relative length of fingers, shortest to longest: II < IV < I < III; tips not expanded; subarticular tubercles prominent, rounded, single; prepollex oval, prominent; two oval, distinct palmar tubercles; supernumerary tubercles absent (Fig. 3A).

Hindlimb heavy and rather short (55.3), about 2.6 times length of forelimb; tibia (16.4) shorter than foot (18.8); heels slightly overlapping when limbs held at right angles to body; tibiotarsal articulation of adpressed limb reaching rear of tympanum; toes long, relative length shortest to longest: I < II < V < III < IV; tips not expanded; toe webs deeply excised, webbing formula: I1–2II1–2<sup>1</sup>/<sub>3</sub>III1<sup>1</sup>/<sub>2</sub>–3IV3–1V; subarticular tubercles prominent, oval (Fig. 3B); ridge of skin on outer edge of fifth toe, from tip of toe to base of metatarsus; inner metatarsal tubercle distinct, oval, length (2.5) about one half of first toe length (4.2); outer metatarsal tubercle low and round; inner tarsal ridge along distal half of tarsus.

Dorsum with irregular skin folds, with pustular warts and granules in between; no dorsolateral fold; supratympanic fold from eye to axilla; side of trunk coarsely granular; ventral side smooth except for coarsely granular posterior half of abdomen; minute colorless asperities on anterior half of throat and breast; distinct nuptial pads, light yellow in color and velvety in structure, covering dorsal and median surfaces of first finger from its base to level of subarticular tubercle; skin on throat side modified; ventrolateral fold from axilla to groin, and sinuous fold across breast.

### Color

In life, dorsum grayish brown with large dark blotches including interorbital bar; no vertebral line medially; upper lip with dark bars; upper half of tympanum surrounded by brown band; limbs marked dorsally with wide and incomplete, dark brown crossbars; rear of thigh with dark blotches; ventrum white with black M-shaped bands across throat; lower lip spotted with blackish brown; ventral surfaces of thigh and tibia whitish (Fig. 1). In preservative, dorsal coloration has slightly faded, but otherwise no obvious change in color or pattern has occurred (Fig. 2).

### Variation

In both Hiroshima and Kumamoto samples, no significant sexual differences were recognized in SVL (ANOVA,  $P > 0.05$ ; Table 1). Statistical comparisons within Hiroshima sample revealed no sexual dimorphisms in all other characters in ratios (Mann-Whitney U tests,  $P > 0.05$ ). Hiroshima and Kumamoto samples, and Kumamoto and Okinawa samples, respectively, did not differ in body proportions in either sex. Okinawa sample had relatively wider upper eyelid than Hiroshima, otherwise the two samples did not differ in any body proportions. Amami sample was slightly distinct, with relatively longer snout and smaller eye than the remaining three samples (Kruskal-Wallis tests with multiple comparisons,  $P < 0.05$ ).

The point reached by the tibiotarsal joint of the hindlimb, bent forward along the body, varied from behind the posterior corner of tympanum to the center of eye, but mostly lies at the point between posterior corners of tympanum and eye in both sexes. Degree of development of toe webbing did not differ sexually. Females lacked black M-shaped bands

across throat, nuptial pads on the first finger, and colorless asperities on ventrum.

From the examination of a larger number of specimens, Kuramoto (1968) reported that populations from three islands of the Amami Islands and Okinawajima Island tended to be larger in SVL and hindlimb length relative to SVL than 10 populations from Kyushu. The light vertebral stripe was present in 44.9–60.9% in Kyushu populations, but was completely absent in the Amami and Okinawa populations (Kuramoto, 1968). There are slight differences in the karyotype between populations from Kyushu and Kikaijima Island of the Amami Islands (Kuramoto, 1971).

### Calls

This species basically has two types of calls, i.e., short and long calls. The short call consists of a series of pulsed notes (Fig. 4). Each of these pulsed notes lasts about 100 ms and is composed of about 10 pulses. The note gap ranges 200–400 ms. The dominant frequency lies at approximately 1200 Hz, and the call has weak frequency modulation and harmonics (Hata and Nagoshi, 1995; Maeda and Matsui, 1999).

### Eggs and larvae

Eggs are laid in several small masses. The mean ( $\pm$  SE) clutch size is  $1244.6 \pm 154.9$  (SE,  $n = 19$ ) and the ovum diameter  $1.13 \pm 0.032$  (from 1.2 to 1.4 mm: Kuramoto, 1978a). The animal hemisphere of egg is light brown in color. Matured larva reaches about 40 mm in total length with a low tail fin and the dental formula of 2(2) / 3 or 2(2) / 3(1). The SVL at metamorphosis is about 14 mm (Maeda and Matsui, 1999).

### Karyotype

Diploid chromosome number is 26, with five large and eight small pairs. Chromosomes forming pairs 4, 8, and 11 are submetacentric and the remaining ten pairs are metacentric. Secondary constrictions are recognized on the shorter arms of pair 7 (Kuramoto, 1971).

### Comparisons

*Fejervarya kawamurai* differs from *F. sakishimensis* in smaller adult SVL (30.7–41.8 mm in males and 36.8–48.7 mm in females vs 45.2–55.5 mm in males and 48.5–69.2 mm in females). In addition, *F. kawamurai* has smaller tympana-

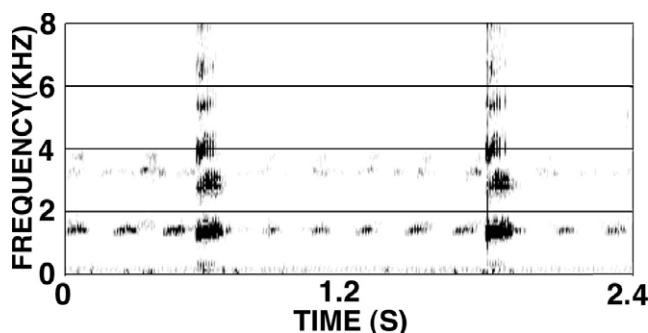


Fig. 4. Sonogram of an advertisement call of *Fejervarya kawamurai* from Kumatori, Osaka, Japan.

num, narrower head, and shorter forelimb, hindlimb, foot, and tibia, all relative to SVL, than *F. sakishimensis*. From *F. multistriata*, *F. kawamurai* differs by relatively shorter forelimb, hindlimb, foot, and tibia. *Fejervarya kawamurai* differs from topotypic samples of *F. limnocharis* and *F. iskandari* in relatively shorter and narrower head and shorter forelimb, hindlimb, foot, and tibia (Table 2).

According to Kotaki et al. (2010), other species phylogenetically grouped into the East-Southeast Asian *Fejervarya* are *F. orissaensis* (Dutta, 1997) and *F. triora* Stuart, Chuaynkern, Chanard, and Inger, 2006. *Fejervarya orissaensis* from India has SVL (34.2–53.8 mm in females and 36.2–47.2 mm in males [Dutta, 1997, as *Limnonectes*]) overlapping *F. kawamurai* (36.8–48.7 mm in females, 30.7–41.8 mm in males), but has a relatively larger tympanum (two thirds [Daniels, 2005, as *Limnonectes limnocharis*] vs one half of eye length in *F. kawamurai*). *Fejervarya triora* from Thailand is larger than *F. kawamurai* in size (54.9–60.2 mm in females), and has broader head and supratympanic fold, with large oval warts on dorsum, in contrast to the long narrow fold in *F. kawamurai*.

### Range

Japan: Honshu, from Kanagawa Pref. and westwards, Shikoku, Kyushu, Amami and Okinawa Island Groups of the Central Ryukyus (Maeda and Matsui, 1999). Artificially introduced into Kanto District of Honshu and Tsushima Is. Possibly in northern and central China and western Taiwan (Fig. 5, see Discussion).

### Natural History

This species generally inhabits plains and hillsides, especially around rice fields. In Honshu, it breeds during May and August in still waters mostly in rice fields, and sometimes in temporary pools. A female lays eggs in small clumps in several times, and is thought to ovulate mature ova little by little during the breeding season (Shichi et al., 1988). Larvae are very tolerant of high temperatures over 40°C (Kuramoto, 1978b). Metamorphosis takes place in late June or later, and most males and many females begin breeding in the following year. It prefers small prey, such as ants, flies, spiders, and earthworms, but sometimes takes frog species (Hirai and Matsui, 2001).

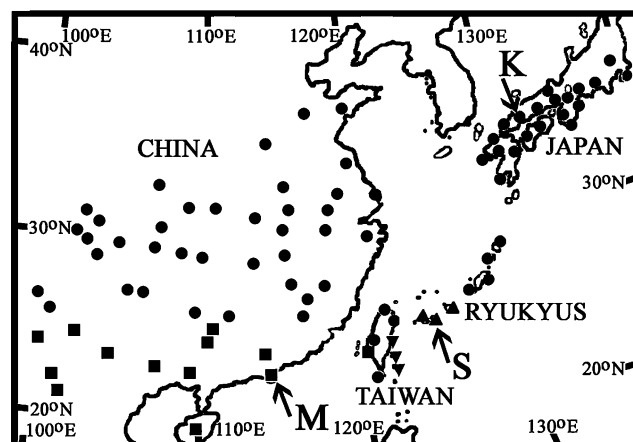
## DISCUSSION

### Japanese Mainland populations

Djong et al. (2007a) carried out artificial crossing between topotypic *F. limnocharis* from Java, Indonesia, and a mainland Japan population from Hiroshima, and found

**Table 2.** Morphometric comparisons of *Fejervarya kawamurai* with congeneric relatives. SVL (mean  $\pm$  1SD in mm) and medians of % ratios of other characters to SVL, followed by ranges in parentheses. Only characters with significant differences ( $P < 0.05$ , shown by \*) from *F. kawamurai* are shown. See text for character abbreviations. M: adult male; F: adult female; J: Juvenile. Three age/sex groups are combined for ratios.

	Species (n)				
	<i>F. kawamurai</i> (17M, 22F)	<i>F. sakishimensis</i> (22M, 16F)	<i>F. multistriata</i> (4M, 13F, 4J)	<i>F. limnocharis</i> (3M, 2F, 3J)	<i>F. iskandari</i> (2M, 4F, 4J)
M SVL	36.9 $\pm$ 3.4 (30.7–41.8)	51.7 $\pm$ 3.0* (45.2–55.5)	42.3 $\pm$ 2.0 (40.4–44.4)	36.8 $\pm$ 1.6 (34.9–37.7)	37.3 (35.6–39.0)
F SVL	42.8 $\pm$ 3.7 (36.8–48.7)	60.6 $\pm$ 5.6* (48.5–69.2)	47.2 $\pm$ 2.9 (44.6–52.4)	45.4 (42.9–47.9)	42.6 $\pm$ 2.5 (39.4–45.2)
RHL	37.3 (35.4–41.1)	37.5 (33.8–40.1)	37.9 (34.9–42.2)	40.1* (37.7–43.4)	39.0* (37.4–40.4)
RTD	6.9 (5.9–8.5)	7.4* (6.3–9.9)	6.9 (5.5–7.5)	7.4 (6.7–8.2)	7.2 (6.2–8.6)
RHW	34.2 (30.3–37.1)	36.8* (34.2–38.6)	35.0 (32.4–38.6)	37.2* (33.7–42.3)	37.1* (35.2–38.7)
RFL	51.7 (37.2–57.6)	58.4* (54.2–64.0)	54.9* (50.6–58.2)	56.0* (52.4–60.6)	57.2* (51.4–61.8)
RHLL	146.1 (126.1–160.4)	161.8* (147.4–175.3)	161.6* (135.4–176.2)	173.5* (158.9–189.5)	173.5* (155.3–192.9)
RTL	43.3 (37.1–48.5)	49.0* (45.2–52.4)	50.3* (41.1–56.1)	52.6* (47.7–58.3)	53.0* (47.2–56.4)
RFL	48.8 (41.9–52.6)	54.0* (49.6–60.7)	53.0* (43.9–58.9)	54.5* (49.1–61.8)	57.2* (50.0–61.6)



**Fig. 5.** Map of East and Southeast Asia showing the distribution of *Fejervarya kawamurai* (closed circles), *F. sakishimensis* (closed triangles), *F. multistriata* (closed squares), and *Fejervarya* sp. (closed inverse triangles). Arrows indicate type localities of *F. kawamurai* (K), *F. sakishimensis* (S), and *F. multistriata* (M). Data from Liu and Hu (1961), Toda et al. (1997, 1998b), Matsui et al. (2007), Zhong et al. (2008), Kotaki et al. (2010), and Matsui (unpublished).

normal larval development to metamorphosis in hybrids. However, Djong et al. (2007b) found these hybrids to have abnormal testes. Together with artificial hybridization, Djong et al. (2007b) conducted morphological observations and phylogenetic analysis through 16S rRNA and Cyt *b* genes, and concluded that the Japanese Mainland (Honshu) population should be regarded as a species distinct from *F. limnocharis*. This led to the description of *F. kawamurai* in this paper.

Nishioka and Sumida (1990) made a genetic study through allozyme variation analyses and found that three western Honshu populations (Hiroshima, Higashihiroshima, and Okayama) were close to each other with small genetic distance ( $D$ ) of 0.009–0.016. Toda et al. (1997) conducted a more extensive allozyme study and also obtained small genetic distances of 0.002–0.118 among 13 populations from three main islands of Japan. Thus genetic variation among the Mainland populations of *F. kawamurai* seems small although detailed studies of genetic variation using DNA sequences have so far never been made.

### Central Ryukyu populations

Kuramoto (1967) reported that all of the artificial hybrids between populations from Kyushu (Fukuoka), Amamioshima and Kikaijima Islands of the Amami Group, and Okinawajima Island of the Okinawa Group were viable and developed into young frogs as normal as the control offspring. Sumida et al. (2002) also obtained similar results between Honshu and Okinawajima populations and concluded that there was no reproductively isolating mechanism between them.

Likewise, close genetic affinity in allozymes between the Mainland (Honshu, Shikoku, and Kyushu) and Okinawajima populations has been reported (Nishioka and Sumida, 1990; Toda et al., 1997). Nishioka and Sumida (1990) obtained  $D$  of the Okinawajima population from three Honshu populations to be 0.030–0.050, which values were much smaller than the  $D$  found between these populations and a Southern Ryukyu population (= *F. sakishimensis*: 0.276–0.387). Toda et al. (1997) found a broader range of variation of  $D$  between Central Ryukyu populations (four from the Amami Group and five from the Okinawa Group) and Mainland populations ( $D$  = 0.017–0.179). These values, however, were again much smaller than  $D$  observed between Central and Southern Ryukyu populations (0.523–0.624).

However, based on the mitochondrial 12S and 16S rRNA genes, Sumida et al. (2002) recognized three clades, each comprising populations from the Japan main islands (Honshu, Shikoku, and Kyushu), the Sakishima-island populations [now *F. sakishimensis*], and the Okinawajima and western Taiwan (Jiayi = Chiayi) populations, respectively. Sumida et al. (2002) surmised the close relationship of Okinawajima and Taiwan populations to be reflecting possible secondary contact between them. As discussed below, Taiwan populations can be genetically split into two distinct lineages, and the Chiayi populations examined by Sumida et al. (2002) belongs to the Western lineage. Thus, their idea applies to the relationships between Central Ryukyu population and Taiwanese populations of the Western lineage.

Similar, but more problematic, results were later obtained by Sumida et al. (2007); In a phylogenetic tree based on short sequence of the 16S rRNA gene, Okinawajima and western Taiwan (Chiayi) populations formed a clade not with Honshu population or *F. sakishimensis* (as Ishigaki and Iriomote populations), but with *F. limnocharis*. As noted above, the populations from the Central Ryukyus slightly differ from Mainland populations like the consistent lack of the mid-dorsal stripe (Kuramoto, 1968). Thus systematic position of the Central Ryukyu populations requires further study.

### Chinese populations

Toda et al. (1997) found close allozyme affinity of Shanghai population with populations from Japan main islands and Central Ryukyus ( $D$  = 0.007–0.117). Another Chinese population from Wenjiang was more distinct from Japan populations ( $D$  = 0.106–0.250), but the difference from the latter was much smaller compared with *F. sakishimensis*. Thus these two populations may be classified as *F. kawamurai* (Fig. 5).

On the other hand, Toda et al. (1998a) suggested the presence of several cryptic species in frogs collectively called *F. limnocharis*, in addition to a distinct biological species in Java, Indonesia (*F. iskandari*). They (Toda et al., 1998a) found that the population from Hongkong (*F. multistriata*) was more remote from the Wenjiang population ( $D$  = 0.250) than to the Laos (Vientiane) population ( $D$  = 0.170). This result suggested the presence of two distinct species, possibly *F. multistriata* and *F. kawamurai*, within China.

Much later, Zhong et al. (2008) treated all the Chinese populations as *F. multistriata* simply following Fei et al. (2002), and investigated the sequencing diversity of the mtDNA D-loop across its distributional area in China. They failed to obtain well-supported phylogenetic trees but recognized two lineages, one occurring around the Yangtze drainage (Yangtze lineage) and another from the south of China (southern lineage), which separation was supported by a nested clade analysis (NCA). The southern lineage defined by Zhong et al. (2008) should be regarded as *F. multistriata* because Hongkong is clearly within the range of that lineage, although they did not sample specimens from Hongkong. Unfortunately, the genetic distance between the two lineages is not available, but the Yangtze lineage may be treated as *F. kawamurai* because the lineage includes the populations from localities close to the ones that showed allozyme affinities with *F. kawamurai* (see above).

### Taiwanese populations

From the study of allozyme variations, Nishioka and Sumida (1990) reported that a population from Chayi (= Chiayi), western Taiwan differed from the Japan Mainland populations with  $D$  of 0.137–0.161. Toda et al. (1997) found allozyme differentiation within Taiwan between one eastern (Taitung, hereafter Eastern lineage) and four western populations (Western lineage;  $D$  = 0.177–0.233). The Western lineage was much more similar to the Japanese and continental Chinese populations ( $D$  = 0.018–0.208) than did Eastern lineage ( $D$  = 0.242–0.333). In contrast, the Eastern lineage was more similar to Southern Ryukyu populations (now *F. sakishimensis*;  $D$  = 0.360–0.414) than was Western lineage (0.485–0.590).

Toda et al. (1998b) further studied this unique genetic differentiation within Taiwan. Among 12 Taiwanese populations, they again found substantial allozyme differentiation between Eastern lineage (eastern main island including Taitung, and Luta and Lanyu) and the Western lineage (northern, western, and southern main island and Penghu Island;  $D$  = 0.129–0.305; Fig. 5). They also confirmed closer similarity to Southern Ryukyu populations (*F. sakishimensis*) in the Eastern lineage ( $D$  = 0.255–0.298) than in the Western lineage (0.367–0.464), as well as much higher similarity to



the continental population from Shanghai in the Western lineage ( $D = 0.019\text{--}0.074$ ) than in the Eastern lineage ( $D = 0.179\text{--}0.273$ ). Toda et al. (1998b) surmised that the Eastern lineage was once subjected to introgression between ancestral genotypes of current Western lineage and *F. sakishimensis*.

However, more recent studies using DNA markers reported results not obtained by the use of allozymes. Zhong et al. (2008) included a population from Taichung (=Taichung), western Taiwan, in their study of the mtDNA D-loop in Chinese populations (see above). Interestingly, the Taichung population had haplotypes of both the Yangtze ( $n = 7$ ) and southern ( $n = 3$ ) lineages (= *F. multistriata*). The haplotype grouped in the southern lineage was close to the Hainan haplotypes, while the haplotypes grouped in the Yangtze lineage were distinct within the lineage.

Kotaki et al. (2010) obtained similar results from the analyses of mitochondrial and nuclear genes. They reported that the eastern Taiwan (Orchard [*sic*, Orchid = Lanyu] and Green [= Luta] Islands) populations, Japan Mainland (Hiroshima) population, and *F. sakishimensis* formed a monophyletic clade, which is sister to another clade encompassing a western Taiwan population from Taipei, *F. multistriata* from Hainan and Husa, and *F. limnocharis*. Kotaki et al. (2010) a priori classified the Taipei population as *F. multistriata*, and because the latter species (*F. multistriata*) as a whole showed 16S rRNA sequence divergence of only 1.1% from *F. limnocharis* and because the latter species (*F. limnocharis*) was nested in the *F. multistriata* clade, they suggested that *F. multistriata* is a junior synonym of *F. limnocharis*. In contrast, they obtained the 16S sequence divergences of 3.3% between eastern Taiwan and the Japan Mainland populations, and 2.7% between eastern Taiwan population and *F. sakishimensis*. They considered these sequence divergences to be nearly equal to the species threshold they defined ( $> 3\%$  for 16S) and suggested heterospecific relationships of eastern Taiwan and the Japan Mainland populations (now *F. kawamurai*; Fig. 5). In this way, systematic relationships of Taiwanese populations of the *Fejervarya limnocharis* complex are far from clear, and much more sampling is needed for elucidating their taxonomic status.

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